15

20

25

30

Inhibition of Matrix Metalloproteinases: Therapeutic Applications (Annals of the New York Academy of Science, Vol 878). Robert A. Greenwald and Stanley Zucker (Eds.), June 1999), and is hereby incorporated by reference.

The phrase "integrin antagonist" includes agents that impair endothelial cell adhesion via the various integrins. Integrin antagonists induce improperly proliferating endothelial cells to die, by interfering with molecules that blood vessel cells use to bridge between a parent blood vessel and a tumor.

Adhesion forces are critical for many normal physiological functions. Disruptions in these forces, through alterations in cell adhesion factors, are implicated in a variety of disorders, including cancer, stroke, osteoporosis, restenosis, and rheumatoid arthritis (A. F. Horwitz, Scientific American, 276:(5): 68-75, 1997).

Integrins are a large family of cell surface glycoproteins which mediate cell adhesion and play central roles in many adhesion phenomena. Integrins are heterodimers composed of noncovalently linked a and b polypeptide subunits. Currently eleven different a subunits have been identified and six different β subunits have been identified. The various a subunits can combine with various b subunits to form distinct integrins.

One integrin known as a_yb_3 (or the vitronectin receptor) is normally associated with endothelial cells and smooth muscle cells. A_yb_3 integrins can promote the formation of blood vessels (angiogenesis) in tumors. These vessels nourish the tumors and provide access routes into the bloodstream for metastatic cells.

The a,b, integrin is also known to play a role in various other disease states or conditions including tumor metastasis, solid tumor growth (neoplasia), osteoporosis, Paget's disease, humoral hypercalcemia of malignancy, angiogenesis, including tumor angiogenesis, retinopathy, arthritis, including rheumatoid arthritis, periodontal disease, psoriasis, and smooth muscle cell migration (e.g. restenosis).

Tumor cell invasion occurs by a three step process:

10 1) tumor cell attachment to extracellular matrix; 2)

proteolytic dissolution of the matrix; and 3) movement

of the cells through the dissolved barrier. This

process can occur repeatedly and can result in

metastases at sites distant from the original tumor.

The a,b, integrin and a variety of other avcontaining integrins bind to a number of Arg-Gly-Asp (RGD) containing matrix macromolecules. Compounds containing the RGD sequence mimic extracellular matrix ligands and bind to cell surface receptors. Fibronectin and vitronectin are among the major binding partners of a,b, integrin. Other proteins and peptides also bind the a,b, ligand. These include the disintegrins (M. Pfaff et al., Cell Adhes. Commun. 2(6): 491-501, 1994), peptides derived from phage display libraries (Healy, J.M. et al., Protein Pept. Lett. 3(1): 23-30, 1996; Hart, S.L. et al., J. Biol. Chem. 269(17): 12468-12474, 1994) and

et al., J. Biol. Chem. 269(17): 12468-12474, 1994) and small cyclic RGD peptides (M. Pfaff et al., J. Biol. Chem., 269(32): 20233-20238, 1994). The monoclonal antibody LM609 is also an a,b, integrin antagonist (D.A.

30 Cheresh et al., J. Biol. Chem., 262(36): 17703-17711, 1987).

5

10

25

A,b, inhibitors are being developed as potential anti-cancer agents. Compounds that impair endothelial cell adhesion via the a,b, integrin induce improperly proliferating endothelial cells to die.

The a_vb₃ integrin has been shown to play a role in melanoma cell invasion (Seftor et al., *Proc. Natl. Acad. Sci. USA*, 89: 1557-1561, 1992). The a_vb₃ integrin expressed on human melanoma cells has also been shown to promote a survival signal, protecting the cells from apoptosis (Montgomery et al., *Proc. Natl. Acad. Sci. USA*, 91: 8856-8860, 1994).

Mediation of the tumor cell metastatic pathway by interference with the a,b, integrin cell adhesion receptor to impede tumor metastasis would be beneficial.

Antagonists of a,b, have been shown to provide a therapeutic approach for the treatment of neoplasia (inhibition of solid tumor growth) because systemic administration of a,b, antagonists causes dramatic regression of various histologically distinct human tumors (Brooks et al., Cell, 79: 1157-1164, 1994).

The adhesion receptor identified as integrin a_vb_3 is a marker of angiogenic blood vessels in chick and man. This receptor plays a critical role in angiogenesis or neovascularization. Angiogenesis is characterized by

the invasion, migration and proliferation of smooth muscle and endothelial cells by new blood vessels. Antagonists of $a_{\nu}b_{3}$ inhibit this process by selectively promoting apoptosis of cells in the neovasculature. The growth of new blood vessels, also contributes to

pathological conditions such as diabetic retinopathy
(Adonis et al., Amer. J. Ophthal., 118: 445-450, 1994)
and rheumatoid arthritis (Peacock et al., J. Exp. Med.,